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Multi-Panel Biomarkers for Diagnosis of Heart Failure Across the Ejection Fraction Spectrum: A Population-Based Study

Mon Myat Oo*,[1] Chuang Gao*,[2] Christian Cole,[2,3] Yoran Hummel,[4] Magalie Guignard-Duff,[2] Adriaan A. Voors,[5] Rudolf A. de Boer,[6] Carolyn S.P. Lam,[7] Jasper Tromp[8] * Chim C. Lang*[1,9]

* Denotes equal contribution

1 Division of Cardiovascular Research, School of Medicine, University of Dundee, United Kingdom

2 Health Informatics Centre, School of Medicine, University of Dundee, United Kingdom

3 Division of Population Health and Genomics, School of Medicine, University of Dundee, United Kingdom

4 Co-founder and president of Us2.ai, the Netherlands

5 University Medical Centre Groningen, Groningen, the Netherlands

6 Erasmus University Medical Centre, Department of Cardiology, Rotterdam, the Netherlands

7 National Heart Centre Singapore, Singapore; Duke-NUS Medical School, Singapore; Singapore.

8 National Heart Centre Singapore, Singapore; Duke-NUS Medical School, Singapore; Saw Swee Hock School of Public Health, National University of Singapore & National University Health System, Singapore.

9. Tuanku Muhriz Royal Chair, Universiti Kebangsaan Malaysia, Bangi, Malaysia

Correspondence:

Chim Lang, Division of Cardiovascular Research, School of Medicine, University of Dundee, Dundee DD1 9SY, United Kingdom

Jasper Tromp, National Heart Centre Singapore, Singapore; Duke-NUS Medical School, Singapore; Saw Swee Hock School of Public Health, National University of Singapore & National University Health System, Singapore. E-Mail: Jasper_tromp@nus.edu.sg

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Abstract

We conducted a cohort study to evaluate the diagnostic value of heart failure (HF) biomarkers across the ejection fraction spectrum. Using electronic health record data from Tayside and Fife regions of Scotland (1993-2021), we linked echocardiograms with plasma samples for biomarker analysis. The study included 156 patients with HF with reduced ejection fraction (HFrEF), 236 patients with preserved ejection fraction (HFpEF), and 186 controls without HF. All biomarkers were significantly elevated in patients with HF compared to controls. Patients with HFrEF had higher hs-TnT, NT-proBNP, and ANG-2 concentrations than those with HFpEF. All nine biomarkers demonstrated strong diagnostic accuracy for distinguishing HF from controls (AUC ≥0.90). However, no two-biomarker pair significantly improved discrimination over its best constituent marker in a majority of splits. This population-based study demonstrated significant diagnostic value of biomarkers for patients with HFrEF and HFpEF. All biomarkers discriminated HF from controls, with held-out AUCs from 0.90 to 0.97. Although each of these biomarkers reflects distinct pathophysiological aspects of HF that could potentially provide complementary diagnostic information, combination of biomarkers in our analysis did not improve discrimination over the best single biomarker. Further work is needed in integrating multi-biomarker panels to enhance patient selection efficiency in clinical trials, improve HF surveillance, and facilitate early diagnosis across healthcare systems, particularly for HFpEF

Introduction

Circulating biomarkers are fundamental for diagnosing heart failure (HF), yet their diagnostic value across the ejection fraction spectrum remains incompletely characterized. Only natriuretic peptides and troponins are currently recommended in international practice guidelines.[1,2] A critical unmet need remains to assess other biomarkers to enhance diagnosis, especially for HF with preserved ejection fraction (HFpEF).[3]

Several novel biomarkers have shown promise in HF, including growth differentiation factor-15 (GDF-15), insulin-like growth factor-binding protein-7 (IGFBP7), bone morphogenetic protein-10 (BMP-10), fibroblast growth factor-23 (FGF-23), dickkopf-related protein 3 (DKK-3), angiopoietin-2 (ANG-2), and Fatty Acid Binding Protein 3 (FABP3) .[4-6] However, few studies have compared diagnostic potential among these biomarkers or have compared this for HF with reduced and mildly reduced ejection fraction (HFrEF/HF(m)rEF) versus HFpEF.[7]

Methods

We used electronic health record data from the Health Informatics Centre (HIC) in Scotland between 1993 and 2021, linking anonymized clinical data with echocardiogram images and biobank plasma samples. HF cases were identified by ICD-10 code I50.x or continuous furosemide prescription.[8] Controls were individuals without HF, furosemide use, or severe valvular disease and with normal/preserved left ventricular ejection fraction. As reported previously [8], we have tested the agreement between patients identified using this identification process by manual validation. The positive predictive value was 86%, 100% sensitivity, 94% specificity with a kappa value of 0.891 was recorded in HFrEF patient groups and the positive predictive value for the diagnosis of HFpEF was 80%, 100% sensitivity, 90% specificity with a kappa value of 0.842. A 100% concordance rate was noted in control group

Patients with HFpEF had documented findings of normal left ventricular ejection fraction (LVEF ≥50%). Patients with HFrEF included those with HF and reduced LVEF (<50%). Comorbidities were identified using ICD codes as previously described.[8] Echocardiographic images were analyzed using an automated deep learning algorithm (Us2.ai, Singapore).

NT-proBNP, hs-TnT and GDF-15 were measured using Roche commercially available IVDs (i.e. Elecsys). BMP-10, IGFBP7, DKK-3, FGF-23, ANG-2 and FABP3 assays are Research Use Only Assays provided by Roche Diagnostics and are only intended for research purposes. They are not approved for clinical use. As previously described for this cohort [8], each plasma sample was matched to the temporally closest echocardiogram, preferentially the study performed before plasma collection, or otherwise the nearest subsequent study within 180 days; no lower limit was applied to echocardiograms preceding the sample. The median interval between the index echocardiogram and plasma sampling was 175 days (IQR 62–442), and 77% of echocardiograms were performed on or before the date of plasma sampling. We also performed a sensitivity analysis restricted to patients whose echocardiogram and blood sample were obtained within 180 days of each other, in which the held-out discrimination of every biomarker was unchanged, indicating that the variable interval between echocardiography and sampling does not account for our findings.

We calculated the area under the curve (AUC) of log-transformed biomarkers with implausible and non-positive values excluded, adjusted for age, sex, eGFR, diabetes, atrial fibrillation, coronary artery disease and COPD, to estimate their discriminatory ability. Analyses were restricted to patients with complete data for the outcome, the biomarker and all covariates. Associations were reported as adjusted odds ratios per standard deviation increase in biomarker level. Discrimination, calibration, and decision thresholds were evaluated using repeated stratified 70/30 split-sample validation over 200 random splits; each metric is summarised by its median across splits with the 2.5th to 97.5th percentile of the split distribution, referred to throughout as the 95% split interval, and calibration was assessed by the calibration slope in the held-out sample. Multiple testing was controlled using the Benjamini-Hochberg false discovery rate, and all analyses were reproducible through fixed random seeds in R. The optimal cut point was based on the Youden Index. Analyses were performed using R Studio (R version 4.5.0).

Results

The study included 156 patients with HFrEF, 236 patients with HFpEF, and 186 controls. Both HF groups were older, had higher body mass index (BMI), and showed increased prevalence of diabetes, chronic obstructive pulmonary disease (COPD), and chronic kidney disease (CKD) compared to controls. Patients with HFpEF were predominantly female and had lower rates of diabetes, atrial fibrillation (AF), and coronary artery disease (CAD) than patients with HFrEF (**Table 1**).

Table 2 shows the coverage and median values with interquartile range (IQR) of the echocardiographic parameters measured by the deep learning workflow. Coverage was generally high, ranging from 46% to 93%. Patients with HFrEF had a higher left ventricular diameter in diastole (LVIDd) than the control and HFpEF group. Patients with HFrEF had a lower LVEF than those with HFpEF and controls. Patients with HFrEF more often had an abnormal left ventricular global longitudinal strain (LVGLS) than those with HFpEF or

controls. Left ventricular filling pressure index E/e' and left atrial end-systolic volume (LAESV) biplane values were higher in HFrEF and HFpEF compared with controls. Compared with controls, patients with HFpEF and HFrEF had greater LV mass, higher mitral E/e' ratio, higher pulmonary artery systolic pressure, more impaired LV strain and right ventricle (RV) dysfunction.

Table 3 shows that all biomarker concentrations were significantly elevated in patients with HF compared to controls. Three biomarkers (hs-TnT, NT-proBNP, and ANG-2) exhibited considerably higher levels in patients with HF(m)rEF compared to those with HFpEF.

Table 4 shows the diagnostic accuracy of the biomarkers for HFrEF and HFpEF. After adjusting for age, sex, eGFR, diabetes, atrial fibrillation, coronary artery disease and COPD, NT-proBNP demonstrated high diagnostic accuracy in differentiating patients with HFrEF/HF(m)rEF from controls (AUC 0.97, 95% split interval 0.93-0.99) and good performance in distinguishing HFpEF from controls (AUC 0.92, 95% split interval 0.88-0.96). These values are for the covariate model with the biomarker added.

All assessed biomarkers demonstrated strong diagnostic performance in distinguishing patients with HF from controls, with held-out AUC values ranging from 0.90 to 0.97 (Table 4).

No two-biomarker pair significantly improved discrimination over its best constituent marker in a majority of splits **(Table 5).** For discriminating HF(m)rEF from HFpEF, NT-proBNP achieved the highest held-out AUC (0.69), with lower concentrations associated with HFpEF (adjusted odds ratio 0.58 per standard deviation, 95% CI 0.44-0.76). Adding hs-TnT to NT-proBNP raised the median held-out AUC for this contrast only marginally, from 0.69 to 0.70, indicating that the combination is not superior to the individual biomarkers.

Discussion

Our findings demonstrate that multiple biomarkers beyond NT-proBNP and troponin have significant diagnostic value in HF. All tested biomarkers demonstrated held-out discrimination between 0.90 and 0.97 for identifying HFrEF and HFpEF from controls.

NT-proBNP remained the most effective single biomarker for distinguishing HF from controls, with higher HFrEF levels than HFpEF. This aligns with previous studies, which have shown that natriuretic peptides are generally lower in HFpEF than in HFrEF, reflecting differences in ventricular wall stress.[9,10]

GDF-15, a member of the transforming growth factor-β family, demonstrated comparable diagnostic accuracy to NT-proBNP in our study. This finding is consistent with Stahrenberg et al., who reported that GDF-15 has an AUC of 0.891 for distinguishing HFpEF from controls, which increased to 0.942 when combined with NT-proBNP.[5] Cardiomyocytes and other cell types express GDF-15 in response to various stressors, exerting anti-inflammatory and anti-apoptotic effects. [11] This suggests that it reflects distinct pathophysiological processes from natriuretic peptides.

Findings for IGFBP7 were consistent with those from the ICON-RELOADED study, which demonstrated that IGFBP7 had a high diagnostic accuracy for acute HF with an AUC of 0.87.[12] Importantly, when combined with NT-proBNP, the diagnostic accuracy improved further with an AUC of 0.94, particularly in patients falling within the "gray zone" of NT-proBNP values.[12] IGFBP7 plays a role in cardiac fibrosis, cellular senescence, and inflammation, processes central to HFpEF pathophysiology.[13] Gandhi et al. demonstrated that IGFBP7 correlates with echocardiographic diastolic dysfunction parameters in HF.[4] These results are consistent with ours, where IGFBP7 was among the strongest single markers for distinguishing HFpEF from controls. However, the absolute increase in diagnostic accuracy was limited.

FABP3 emerged as another biomarker with strong diagnostic capability in our study. This intracellular protein is released during myocardial injury and may reflect ongoing cardiomyocyte damage, even in the absence of acute coronary syndromes. The high sensitivity of FABP3 for cardiac injury makes it particularly valuable for the early detection of HF-related myocardial damage.[14]

The distinctive biomarker profiles between HFrEF and HFpEF further highlight the different pathophysiological mechanisms underlying these conditions. Notably, hs-TnT, NT-proBNP, and ANG-2 concentrations were higher in patients with HFrEF than HFpEF, reflecting potentially greater myocardial stress and injury in HFrEF.[15]

This study's strengths include its population-based design, the use of linked electronic health records with biospecimens, and the application of deep learning for echocardiographic analysis. Limitations include the reliance on EHR-based definitions and the study's observational nature. Since the number of participants was limited, our results require larger cohort participants studies for validation. Future studies will benefit from new insights derived from our better understanding of the pathophysiology of HF.[16] Finally, our study was not able to provide any mechanistic insights into why certain biomarkers differ between HFrEF and HFpEF. Single-cell technologies such as single-cell RNA sequencing may offer a route to resolving these mechanisms and may in turn refine biomarker-based diagnosis of HF.[17]

In terms of the use of biomarkers for the diagnosis and prognostication of HF, it is important to distinguish between biomarkers that are currently established in clinical practice and those that remain largely investigational. Of the biomarkers evaluated in our study, NT-proBNP and hs-TnT can be measured using commercially available, standardised diagnostic assays and are already well established in clinical practice, particularly for the diagnosis, risk stratification, and prognostication of patients with heart failure. GDF-15 can also be measured using commercially available assays and has been evaluated extensively in clinical studies; however, its clinical use remains considerably less widespread, and it is not currently part of routine heart failure assessment.

In contrast, the other biomarkers investigated in our study, including BMP-10, IGFBP7, DKK-3, FGF-23, ANG-2, and FABP3, are not currently established as routine clinical biomarkers and the assays used to measure them remain primarily research-based. Although these biomarkers have demonstrated potential diagnostic or prognostic value in research settings, their clinical utility has yet to be established. In particular, there remain important questions regarding assay standardisation, analytical performance, reproducibility across different populations and laboratories, appropriate reference ranges, and the incremental value of these biomarkers beyond established clinical variables and currently available biomarkers.

Therefore, while our findings provide further evidence supporting the potential relevance of these biomarkers in heart failure, they should not be interpreted as demonstrating immediate clinical applicability. Further prospective studies and external validation are required to determine whether these biomarkers provide clinically meaningful information beyond established risk factors and routinely available biomarkers such as NT-proBNP and hs-TnT. Ultimately, demonstrating feasibility, reproducibility, cost-effectiveness, and incremental clinical value will be essential before these emerging biomarkers can be translated into routine clinical practice.

Conclusion

This population-based study demonstrated significant diagnostic value of multiple biomarkers for patients with HFrEF and HFpEF. All biomarkers discriminated HF from controls, with held-out AUCs from 0.90 to 0.97. Although each of these biomarkers reflects distinct pathophysiological aspects of HF that could potentially provide complementary diagnostic information, combination of biomarkers in our analysis did not improve discrimination over the best single biomarker. Further work is needed in integrating multi-biomarker panels to enhance patient selection efficiency in clinical trials, improve HF surveillance, and facilitate early diagnosis across healthcare systems, particularly for HFpEF.

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Table 1: Demographic and clinical characteristics of the study groups

	Control	**HFrEF**	**HFpEF**	**p-value[a]**
Patients (n)	186	156	236	
Demography				
Age, years (median±SD)	59.5±18	74±10	77.5±13	<0.001
Sex, female (%)	61%	37%	61%	<0.001
BMI[b]	25.8±6.4	27.0±0.4	28.9±5.8	0.87
Medical history (%)				
Diabetes	9%	39%	31%	<0.001
AF	NA	40%	30%	<0.001
CAD	NA	46%	29%	<0.001
COPD	32%	53%	56%	<0.001
CKD	3%	42%	39%	<0.001
Laboratory				
Serum creatinine (umol/L)	67±15.3	93±98.1	83±88.4	<0.001
eGFR (MDRD) (mL/min/1.73m^2)	94±27.7	63.6±23.6	66.9±27.2	<0.001
Potassium (mmol/L)	4.3±0.4	4.4±0.4	4.3±0.5	0.68
Sodium (mmol/L)	139±3.2	139±3.3	139±3.7	0.36
Medication (%)				
Furosemide	NA	90%	83%	<0.001
Bumetanide	NA	13%	11%	<0.001
Spironolactone	2%	31%	19%	<0.001
Eplerenone	NA	17%	3%	<0.001
Aspirin	24%	68%	59%	<0.001
Statin	25%	78%	69%	<0.001
SGLT2I	1%	2%	2%	0.48
Betablocker	31%	93%	77%	<0.001

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ARB	14%	33%	33%	<0.001
ACEI	20%	82%	68%	<0.001

a. p-value is calculated using either ANOVA or Chi-squared test. Chi-squared test is used when the data is categorical, e.g., sex, comorbidity, and medication.

b. Based on 43 patients with weight and height available

Abbreviations: BMI =body mass index, AF=atrial fibrillation, CAD=coronary artery disease, COPD= chronic obstructive pulmonary disease, CKD=chronic kidney disease, eGFR=estimated glomerular filtration rate, MDRD = Modification of Diet in Renal Disease, NT-proBNP=n terminal pro B type natriuretic peptide, SGLT2i= sodium-glucose co-transporter-2 inhibitors, ARB= Angiotensin receptor blockers, ACEI= Angiotensin-converting enzyme (ACE) inhibitors,

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Table 2.
Echocardiographic characteristics of the study groups (parameters derived from the Us2.ai software analyses) (median ± SD)

	Us2.ai features	**Coverage**	**Control**	**HFpEF**	**HFrEF**	***P*-value**
LV dimensions (linear)	IVSd (mm)	93%	8.7 ± 1.8	9.8 ± 2.3	9.8 ± 2.2	<0.001
	LVIDd (mm)	93%	45.8 ± 5.7	44.9 ± 6.1	51.5 ± 7.3	<0.001
	LVPWd (mm)	92%	8.4 ± 1.2	9.4 ± 1.6	9.3 ± 1.6	<0.001
	LVIDs (mm)	91%	30.9 ± 5.7	32.2 ± 7.3	42.8 ± 8.5	<0.001
	RWT (mm)	93%	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	<0.001
	LV mass (g)	93%	123 ± 40.4	150.8 ± 43.4	183 ± 50.4	<0.001
LV (volume and) systolic function	LVEF (any single plane) (%)	92%	62.7 ± 9.3	59.6 ± 11	41.6 ± 15.5	<0.001
	LVEF biplane (%)	73%	62.5 ± 8.5	59.8 ± 10.2	41.4 ± 14.3	<0.001

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	Us2.ai features	Coverage	Control	HFpEF	HFrEF	*P*-value
	LVEDV biplane (mL)	74%	79.6 ± 23.9	81.9 ± 30.7	109.6 ± 42.7	<0.001
	LVESV biplane (mL)	73%	30.3 ± 12.3	33.7 ± 17.7	63.7 ± 36.1	<0.001
LVGLS	LVGLS (overall)	60%	−21.2 ± 3.4	−18.4 ± 4.3	−12.1 ± 4.3	<0.001
	LVGLS (apical 3-chamber)	73%	−21.6 ± 5.1	−17.8 ± 6.5	−11.9 ± 5.7	<0.001
	LVGLS (apical 4-chamber)	91%	−20.9 ± 4.1	−19.1 ± 4.9	−12.2 ± 4.8	<0.001
	LVGLS (apical 2-chamber)	78%	−21.5 ± 4.4	−19 ± 5.7	−12.8 ± 5.1	<0.001
LV diastolic function	MV E (cm/s)	88%	71.2 ± 17.5	79 ± 33.7	88.5 ± 30.3	<0.001
	MV A (cm/s)	71%	69.7 ± 20.9	82.8 ± 28.4	73.7 ± 29.2	<0.001
	E/A ratio	70%	1 ± 0.4	0.8 ± 0.5	1 ± 0.6	0.059
	DT (ms)	76%	223.2 ± 46.6	209.8 ± 64.6	180.4 ± 65	<0.001
	e′ septal (cm/s)	52%	7.4 ± 3	5.9 ± 2	4.8 ± 2.1	<0.001
	e′ lateral (cm/s)	52%	10.6 ± 4	8.1 ± 2.6	7.7 ± 2.8	<0.001
	e′ mean (cm/s)	47%	9 ± 3.1	7.2 ± 2	6.3 ± 2	<0.001
	E/e′ mean (cm/s)	46%	7.7 ± 2.9	10.3 ± 4.9	12.6 ± 5.6	<0.001
	LAESV biplane (mL)	59%	35.9 ± 15.2	52.4 ± 27.2	59.3 ± 25.6	<0.001
RV function	TAPSE (mm)	75%	22.7 ± 5	21 ± 5.1	18.7 ± 4.7	<0.001
	RVFAC (%)	57%	36.2 ± 13	30.4 ± 13.2	26.3 ± 15.4	<0.001
	RVIDd (mm)	76%	33.4 ± 5.6	35.4 ± 6.3	36.2 ± 7.4	<0.001
	TR Vmax (m/s)	68%	2.3 ± 0.4	2.5 ± 0.6	2.7 ± 0.6	<0.001

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	Us2.ai features	Coverage	Control	HFpEF	HFrEF	*P*-value
	TR Pmax (mmHg)	73%	17.9 ± 6.6	21.9 ± 14.9	23.7 ± 9.1	<0.001
RA size	RAESV (mL)	80%	29.9 ± 18.6	38.1 ± 23.7	41.1 ± 33.6	<0.001
	RA area (cm^2/m^2)	72%	12.8 ± 3.8	15.6 ± 5	15.7 ± 6.4	<0.001

DT, deceleration time; E/A, mitral valve early diastolic velocity to late diastolic velocity ratio; E/e', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; IVSd, interventricular septum thickness in diastole; LAESV, left atrial end-systolic volume; LV, left ventricle; LVGLS, left ventricle global longitudinal strain; LVEDV, LV end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVIDd, left ventricular internal. Diameter at end-diastole; LVIDs, left ventricular internal diameter end-systole; LVMass, left ventricle mass; LVPWd, end-diastolic left ventricular posterior wall thickness; MV A, mitral valve late diastolic velocity; MV E, mitral valve early diastolic velocity; PASP, pulmonary arterial systolic pressure; RA area, right atrial area; RAESV, right atrial end-systolic volume; RV.FAC, right ventricular fractional area changes; RVIDd, right ventricular internal dimension at end-diastole; RWT, relative wall thickness; TAPSE, tricuspid annular plane systolic excursion; TR Pmax, tricuspid regurgitation maximum pressure; TR Vmax, tricuspid regurgitation maximum velocity.

Table 3: Biomarker Concentrations Across Study Groups (Median [Q1-Q3])

Biomarker	**Controls (non-HF) n=186**	**HFrEF n=156**	**HFpEF n=236**
hs-TnT (pg/mL)	3.9 [3-8.4]	21.5 [11.8-42.3]	16.4 [9.2-28.2]
GDF-15 (pg/mL)	1356 [901.6-2056]	3376 [1940-6463.8]	3036.5 [2048.2-4985.8]
NT-proBNP (pg/mL)	77.2 [32.8-155.8]	1637 [520.6-4451.2]	686.3 [203-1984.2]
IGFBP7 (ng/mL)	75.2 [63-87]	113.2 [89.9-151.8]	102.3 [86.5-136.5]
BMP-10 (ng/mL)	1.7 [1.4-2]	2.2 [1.8-2.8]	2.1 [1.7-2.7]
DKK-3 (ng/mL)	39.4 [31.4-50.2]	58.5 [46.6-72.2]	53 [43.8-68.7]
FGF-23 (pg/mL)	127.1 [94.5-199.8]	227.5 [142.9-554.4]	210.1 [140.1-500.1]
ANG-2 (ng/mL)	1.4 [1.1-2]	2.9 [1.8-5.7]	2.4 [1.7-4]

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FABP3 (ng/mL)	21.4 [17.1-27.4]	37.8 [28.6-60.7]	36.3 [27.2-56.9]

Table 4: Adjusted association and held-out discrimination of each biomarker, by pairwise contrast

Comparison	Biomarker	N	OR_perSD	OR_q	AUC
Control vs HFpEF	hs-TnT (pg/mL)	414	2.26 (1.55-3.28)	5.79e-05	0.91
	GDF-15 (pg/mL)	409	1.75 (1.22-2.52)	0.00372	0.91
	NT-proBNP (pg/mL)	414	2.88 (1.88-4.4)	9.99e-06	0.92
	IGFBP7 (ng/mL)	415	2.46 (1.5-4.02)	0.00062	0.91
	BMP-10 (ng/mL)	415	1.5 (1.06-2.12)	0.02340	0.91
	DKK-3 (ng/mL)	414	1.31 (0.92-1.88)	0.13400	0.91
	FGF-23 (pg/mL)	403	2.15 (1.48-3.12)	0.00014	0.91
	ANG-2 (ng/mL)	412	2.7 (1.79-4.08)	1.11e-05	0.93
	FABP3 (ng/mL)	406	1.69 (1.1-2.58)	0.02030	0.9
Control vs HFrEF	hs-TnT (pg/mL)	325	2.24 (1.2-4.2)	0.01760	0.96
	GDF-15 (pg/mL)	330	2.1 (1.23-3.61)	0.01270	0.95
	NT-proBNP (pg/mL)	331	4.3 (2.18-8.49)	0.00023	0.97
	IGFBP7 (ng/mL)	328	2.75 (1.34-5.68)	0.01270	0.96
	BMP-10 (ng/mL)	327	2.03 (1.21-3.39)	0.01270	0.96
	DKK-3 (ng/mL)	328	1.73 (1.04-2.89)	0.03870	0.95
	FGF-23 (pg/mL)	314	1.93 (1.1-3.4)	0.02840	0.95
	ANG-2 (ng/mL)	327	2.68 (1.5-4.8)	0.00396	0.96
	FABP3 (ng/mL)	320	1.86 (0.97-3.56)	0.06000	0.95
HFrEF vs HFpEF	hs-TnT (pg/mL)	371	1.04 (0.78-1.39)	0.86800	0.65
	GDF-15 (pg/mL)	389	0.98 (0.75-1.28)	0.90400	0.64

	NT-proBNP (pg/mL)	377	0.58 (0.44-0.76)	0.00097	0.69
	IGFBP7 (ng/mL)	381	1.07 (0.8-1.41)	0.86800	0.63
	BMP-10 (ng/mL)	380	0.85 (0.67-1.07)	0.37600	0.63
	DKK-3 (ng/mL)	380	0.88 (0.7-1.12)	0.56200	0.63
	FGF-23 (pg/mL)	357	1.04 (0.81-1.32)	0.86800	0.63
	ANG-2 (ng/mL)	379	0.78 (0.62-0.98)	0.14200	0.65
	FABP3 (ng/mL)	366	1.31 (0.96-1.79)	0.27800	0.66

Footnote for Table 4:

a. *Abbreviations: High-sensitivity Troponin (hs-TnT), Growth/differentiation factor-15 (GDF-15), NT-pro B type natriuretic peptide (NT-proBNP), IGFBP7 (Insulin-Like Growth Factor Binding Protein 7), BMP-10 (Bone Morphogenetic Protein 10), Dickkopf 3 (DKK-3), Fibroblast growth factor-23 (FGF-23), Angiopoietin 2 (ANG-2), Fatty Acid Binding Protein 3 (FABP3).*
b. *For HF(m)rEF vs HFpEF, HF(m)rEF = 0 vs HFpEF = 1, so an odds ratio above 1 indicates a more HFpEF-like profile.*
c. *N is the number of patients analysed after exclusion of biomarker values outside Q1 − 10×IQR to Q3 + 10×IQR and restriction to observations complete for the outcome, the biomarker and all seven covariates. OR_perSD is the adjusted odds ratio per one standard deviation of $\log_{10}$(biomarker) with 95% confidence interval; it is scaled per standard deviation rather than per 10-fold because the biomarkers differ by orders of magnitude in dynamic range. OR_q is the Benjamini-Hochberg false discovery rate adjusted p-value for that odds ratio. AUC is the held-out area under the ROC curve for the covariate model with the biomarker added.*

Table 5: One Row Per Pairwise Contrast, Summarising All Two-biomarker Combinations

Comparison	N_pairs_tested	Best_single	AUC_best_single	Best_pair	AUC_best_pair
Control vs HFpEF	36	ANG-2 (ng/mL)	0.93	hs-TnT (pg/mL) + ANG-2 (ng/mL)	0.93
Control vs HFrEF	36	NT-proBNP (pg/mL)	0.97	hs-TnT (pg/mL) + GDF-15 (pg/mL)	0.96
HFrEF vs HFpEF	36	NT-proBNP (pg/mL)	0.69	hs-TnT (pg/mL) + NT-proBNP (pg/mL)	0.7

Footnote for Table 5:

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a. *N_pairs_tested is the number of two-biomarker combinations evaluated, that is all 36 pairs of the nine biomarkers. Best_single and Best_pair are the single biomarker and the pair achieving the highest median held-out AUC within that contrast, with AUC_best_single and AUC_best_pair giving those values.*

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Graphical Abstract

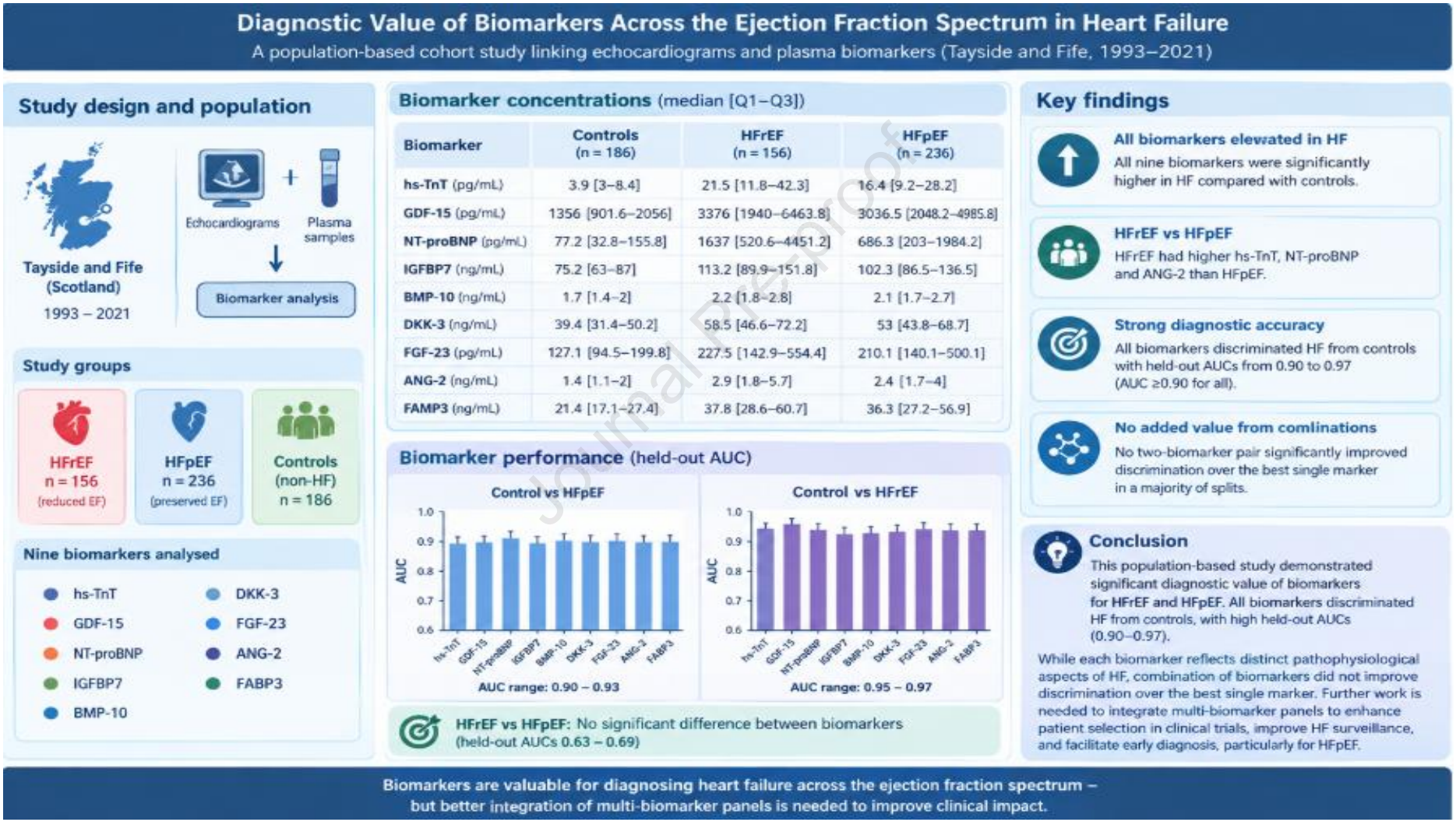
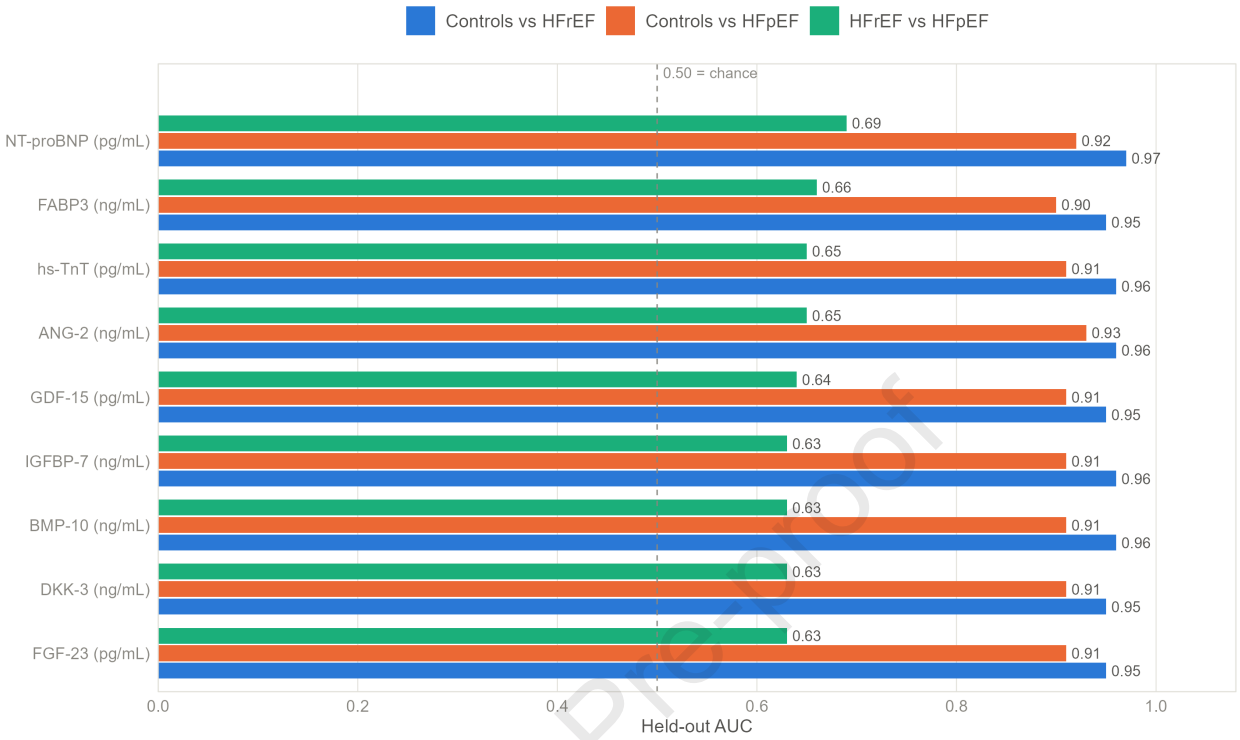


Figure 2. Held-out discrimination (AUC) of each biomarker, by pairwise contrast

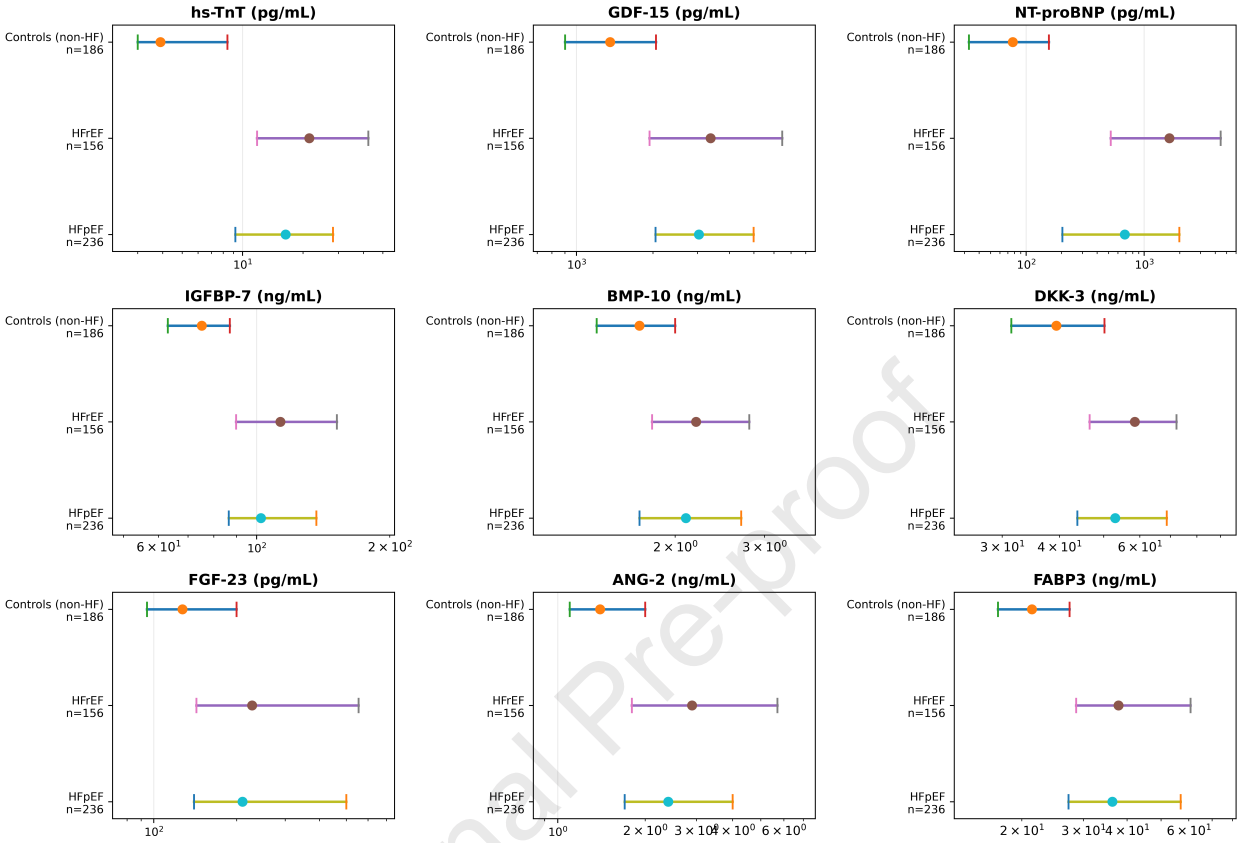
Every biomarker separates heart failure from controls (AUC 0.90-0.97), but none separates HFrEF from HFpEF (AUC 0.63-0.69)



AUC is the held-out area under the ROC curve for the covariate model (age, sex, eGFR, diabetes, atrial fibrillation, coronary artery disease, COPD) with the biomarker added. Biomarkers are ordered by AUC for the HFrEF vs HFpEF contrast. Dashed line marks AUC 0.50 (no discrimination). Values from Table 4.

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Figure 1. Biomarker Concentrations Across Study Groups



Points represent median concentrations; horizontal lines represent interquartile ranges (Q1–Q3). Each biomarker is displayed on a log-scaled x-axis.

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Highlights

- Electronic health records linked with echocardiogram and plasma sample for biomarker analysis
- Three groups of patients such as heart failure with reduced ejection fraction, heart failure with preserved ejection fraction and control patients without heart failure were identified.
- Biomarkers such as hs-TnT, GDF-15, NT-proBNP, IGFBP7, BMP-10, FGF-23, Dkk3, ANG-2 and FABP3 were analyzed
- All tested biomarkers were significantly elevated in patients with HF compared to controls.
- HFrEF patients had higher hs-TnT, NT-proBNP, and ANG-2 concentrations than HFpEF patients.
- NT-proBNP, hs-TnT, GDF-15, IGFBP7, and FABP3 showed strong diagnostic accuracy (AUC >0.8) for distinguishing HF from controls.

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